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Do predators affect the survival of *Macaria pallidata* larvae? Implications for biological control of *Mimosa pigra* in the NT

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Abstract

There have been few studies investigating whether predators can affect the survival of insects that have been introduced into new regions. To address this, ants and birds were excluded from mimosa (*Mimosa pigra*) plants that had larvae of a leaf-feeding geometrid moth, *Macaria pallidata* placed on them. The moth is used as a biological control agent against mimosa in the Northern Territory. More larvae were observed when ants and birds were excluded. The ants present were generalists, probably attracted to mimosa by the nectar it supplies at the base of the leaves.

Introduction

Few studies have investigated factors influencing the establishment of insect populations introduced into new areas. Failure of an insect to colonise a new area is commonly attributed to climatic variables, often without any data to support such a claim (Clarke 2001). The actual causes of failure are rarely studied, but can have important implications for biological control programs (Day *et al.* 2004). Such knowledge should be used when selecting future biological control agents, considering the large costs associated with finding, testing and introducing them.

The macaria moth *Macaria pallidata* (Lepidoptera, Geometridae) is a biological control agent released against the weed mimosa (*Mimosa pigra* L) in Australia. Macaria was identified as a potential biological control agent against mimosa, but was originally ignored because it was considered too vulnerable to predators and parasitoids (Harley *et al.* 1995).

Female macaria lay eggs on leaves and stems, and larvae feed for c. 13 days. Larvae are soft-bodied, slow moving "looper" caterpillars that grow up to 2 cm long and feed externally on mimosa leaves (Heard *et al.* 2001). Larvae go through five instars then form prepupae, which are obviously shorter, thicker and darker coloured. Most larvae descend to the ground to pupate. Larvae drop on a silken thread when disturbed (Heard *et al.* 2001, B. Routley, pers. comm.).

Mimosa has been the target of a large, ongoing biological control program since 1979 (Paynter & Flanagan 2002). It is an invasive weed that infests approximately 800 km^2 of Top End floodplains, and has the potential to spread throughout much of tropical Australia (Walden *et al.* 2002). To date, twelve species of insects and two species of fungi have been released against this weed. Seven of these insect species have established (N. Ostermeyer, unpubl.). Macaria was the first insect released that primarily attacks mimosa leaves. Mimosa in the Northern Territory now grows more slowly and produces considerably less seed than it did before biological control (Paynter & Flanagan 2002, Paynter in press).

Initial releases of macaria in Australia (between June 2002 and December 2004) appeared to be unsuccessful – no insects could be found at release sites, despite extensive searches (B. Routley, unpubl.). Predators such as ants, spiders and birds have been observed eating and disturbing caterpillars in the Top End, and birds have also been observed eating macaria adults. This experiment aimed to determine if ants and bird predators do influence survival of macaria larvae.

Methods

The experiment was conducted near Beatrice Hill Lagoon (12° 33' S, 130° 18' E), on the Adelaide River floodplain. The site was previously dominated with spike-rushes (*Eleocharis* spp.) (Story 1969), and much of the area was invaded by mimosa in the 1970s (Braithwaite *et al.* 1989). Since 1989 the inundated areas have been taken over by the introduced pasture grasses *Hymenachne amplexicaulis* and *Brachiaria mutica*, leaving little *Eleocharis* remaining.

Mimosa planted in 1999 was used for the experiment. These plants had been planted for previous experiments, and were spaced 3 m apart. To make plants more homogeneous and improve the probability of finding insects, plants were trimmed to *c.* 1.5 m high one month before the experiment commenced. To ensure the ant exclusion treatment was effective, all vegetation touching each plant was removed.

Four treatments were applied randomly to mimosa plants:

1. Ants were excluded by manually removing all ants seen, then applying sticky gel ("Tac-gel", Rentokil) around the base of each plant. This gel was inspected several times, and any large sticks or leaves removed. There were six replicate plants used in this treatment.

2. Birds were excluded with commercial bird netting, which was set up around the plants but not touching foliage. It was not known which bird species were likely to eat larvae (eight replicate plants).

3. Plants had both ants and birds excluded (seven replicate plants).

4. A control group was left untouched (seven replicate plants).

Fifty larvae (third to fifth instar) were placed randomly on the foliage of each plant on 7 November 2002. Larvae on each plant were counted for 5 minutes on 8, 11 and 13 November. The experiment was terminated on 13 November, when no larvae were observed and all larvae would have pupated. Two observers did all the larvae counting, and were assigned plants at random. To test for differences between observers, both observers counted larvae on 12 plants on 8 November.

A sample of ants from each plant was collected on 6 November and identified soon after. There was no specific survey of bird species present in the area.

The number of larvae counted on 8 and 11 November were compared between treatments using generalised linear models with Poisson error distribution, after first removing effects of the person counting larvae by treating this as an additional variable, and checking for overdispersion.

Results

One day after placing larvae on plants, more larvae were found on plants where ants had been excluded (Figures 1, 2, d.f. = 1, $\chi^2 = 32$, $p < 0.0001$). Excluding birds also affected larval survival (d.f. = 1, $\chi^2 = 3.8$, $p = 0.009$). There was no important interaction between excluding birds and excluding ants (d.f. = 1, $\chi^2 = 1.0$, $p = 0.3$).

Figure 1. Number of larvae counted per plant, one day after larvae were placed on mimosa plants. Plants had either no predators excluded (control) or ants, birds, or both excluded. Raw data points (♦) are staggered on the x-axis so all data points can be shown, and horizontal bars show the mean for each treatment.

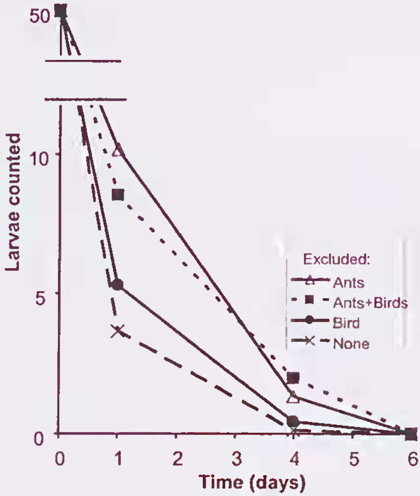

Figure 2. Number of larvae counted per plant, over time, after 50 larvae were placed on each plant at day 0. Plants had either ants (Δ), birds (•), both (■), or no predators excluded (X).

Excluding ants increased the number of larvae found (d.f. = 1, $\chi^2 = 20$, $p < 0.0001$) four days after placing larvae in the field (Figure 2). Excluding birds appeared to slightly affect larval survival (Figure 2), although this was not significant (d.f. = 1, $\chi^2 = 2.9$, $p = 0.08$). There was no important interaction between excluding birds and excluding ants (d.f. = 1, $\chi^2 = 0.9$, $p = 0.3$). Numbers of larvae observed decreased over time, with more larvae observed on plants with ants excluded (Figure 2). No larvae were observed on day 6.

Overall, few larvae were found, and ants were observed disturbing larvae, which then descended to the ground on a silk thread. There were differences between observers (d.f. = 1, $\chi^2 = 4.4$, $p = 0.03$), which suggests that the larval counting process may not have been perfect and not all larvae present were counted. Only one larva was found caught in the sticky gel and 21 prepupae were found; 12 in the ant-excluded plants, and 9 in plants that had ants and birds excluded.

The ants collected were *Polyrhachis crawleyi*, *P. schenkii*, *P.* sp. nr *obtusa*, *Rhytidoponera* sp. nr *aurata*, *Odontomachus* sp. nr *turneri*, *Crematogaster* sp. (*C. laeviceps* group) and *Ochetellus* sp. Very little is known about the ecology of these species, but all ants appear to be generalist scavengers rather than specialised predators (A. Andersen, pers. comm.). Within two days of excluding ants, plants had obvious large, sticky globules exuding from the extrafloral nectaries at the base of each leaf.

Crimson Finches *Neochmia phaeton* were observed near the mimosa plants, and twice were found trapped inside the bird nets; these are however primarily seed-feeders (Todd *et al.* 2003). The only insectivorous birds observed in the area were Rainbow Bee-eaters *Merops ornatus*.

Discussion

Ants commonly disturbed macaria larvae, and either removed them or caused them to drop on silk threads. Birds had an effect on larval numbers, but this was not as important as the effect of ants.

Low numbers of larvae were found after release. It is possible that many larvae were present in the foliage but not found. The cryptic colour of the larvae and differences in larvae counted on the same plants between observers suggests this is likely. Many larvae probably also pupated within several days of being placed on plants.

Ant species found on mimosa were generalists. Given that large sticky globules were observed at the base of the leaves when ants were excluded, we can conclude thar ants feed from these extrafloral nectaries. It is likely that mimosa evolved these nectaries to attract such ants, which then deter herbivores, as is common in the Mimosaceae outside of Australian rangelands (Norris *et al.* 1994); these associations appear to be common in the native range of mimosa. Of the leguminous plants studied in Mexico, 73% had close associations with ants, and use of extrafloral nectaries was the most common ant-plant interaction in the native range of mimosa (Rico-Gray *et al.* 1998). This association with a range of ants that deter herbivores may be one reason why mimosa has become such a 'successful' weed.

The sticky gel may have also deterred other predators such as spiders, frogs and lizards. Being a wetland, the area has an abundance of frogs, such as *Litoria bicolor*, which commonly sits on mimosa branches (pers. obs.). These other predators were not investigated in this study, but none were observed caught in the gel.

Overall, only a small proportion of all larvae placed on mimosa plants were seen. Larvae used in this experiment were late instar, so many may have dropped to the ground to pupate during the trial. Larvae are also cryptic, and some may have been missed while counting.

When this experiment was conducted, macaria had not been recovered from any site where it had been released. Recent surveys, however, have found that it has established and spread widely on mimosa in the Northen Territory (B. Routley, unpubl.). Although predators such as ants and birds do remove and disturb a considerable proportion of larvae, macaria is still capable of surviving and spreading. This may be due in part to the insect's high fecundity (Heard *et al.* 2001), or because the density of mimosa thickets would allow larvae to simply drop onto a lower branch if disturbed. The effect predators have on population density is still unknown. This paper shows that predators can remove considerable numbers of larvae, although this does not necessarily prevent insects from colonising new areas.

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